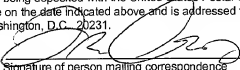
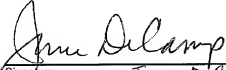


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TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		U.S. Application Number: 097445362
INTERNATIONAL APPLICATION NUMBER	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/EP 98/03584	15/06/1998	13/06/1997
TITLE OF INVENTION:	MYOCARDIUM- AND SKELETAL MUSCLE-SPECIFIC NUCLEIC ACID, ITS PREPARATION AND USE	
APPLICANTS FOR DO/EO/US:	Marion Elke Hofmann, Horst Domdey, and Thomas Henkel	
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:		
1.	<input checked="" type="checkbox"/>	This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.
2.		This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.
3.	<input checked="" type="checkbox"/>	This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4.	<input checked="" type="checkbox"/>	A proper Demand for International Preliminary Examination was made by the 19 th month from the earliest claimed priority date.
5.		A copy of the International Application as filed (35 U.S.C. 371(c)(2)).
a.		is transmitted herewith (required only if not transmitted by the International Bureau).
b.	<input checked="" type="checkbox"/>	has been transmitted by the International Bureau.
c.		Is not required, as the application was filed with the United States Receiving Office (RO/US).
6.	<input checked="" type="checkbox"/>	A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7.		Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)).
a.		are transmitted herewith (required only if not transmitted by the International Bureau).
b.		have been transmitted by the International Bureau.
c.		have not been made; however, the time limit for making such amendments has NOT expired.
d.	<input checked="" type="checkbox"/>	have not been made and will not be made.
8.		A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9.	<input checked="" type="checkbox"/>	An unsigned oath or declaration of the inventors (35 U.S.C. 371(c)(4)).

10.	A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).		
11.	An Information Disclosure Statement under 37 CFR 1.97 and 1.98.		
12.	An assignment for recording. A separate cover sheet in compliance with 37.3.28 and 3.31 is included.		
13.	A FIRST preliminary amendment.		
	A SECOND or SUBSEQUENT preliminary amendment.		
14.	A substitute specification.		
15.	A change of power of attorney and/or address letter.		
16.	X	Other items or information: PCT Request (PCT Antrag) and PCT International Search Report (Formblatt PCT/ISA/210)	
17.		<p>The following fees are submitted:</p> <p>BASIC NATIONAL FEE (37 CFR 1.492(A)(1)-(5)):</p> <p>Search Report has been prepared by the EPO or JPO \$ 930.00</p> <p>International preliminary examination fee paid to USPTO (37 CFR 1.482) \$ 720.00</p> <p>No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee (37 CFR 1.445(a)(2)) paid to USPTO \$ 790.00</p> <p>Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO \$ 1070.00</p> <p>International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) \$ 98.00</p>	\$1070.00
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CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE
Total claims	[**] - 20 =		x \$22.00 \$
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TOTAL NATIONAL FEE =		\$	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property.		+ \$	
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SEND ALL CORRESPONDENCE TO:		 Signature <i>James DeLamp</i> Karen L. Elbing, Ph.D. Reg. No. 43,580 Reg No. 35,238	
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**Myocardium- and skeletal muscle-specific nucleic acid,
its preparation and use**

5

The invention relates to a nucleic acid which is expressed in human myocardium and skeletal muscle, to its preparation and use as diagnostic aid, medicinal product and test for identifying functional interactors.

10

The heart is a muscular hollow organ which has the task of keeping the bloodstream in the vessels in motion by alternating contraction (systole) and relaxation (diastole) of atria and ventricles.

15

The muscle of the heart, the myocardium, is composed of specialized striped muscle cells between which there is connective tissue. Each cell has a central nucleus, is bounded by the plasma membrane, the sarcolemma, and contains numerous contractile myofibrils which are separated irregularly by sarcoplasm. The contractile substance of the heart is formed by long parallel myofibrils. Each myofibril is divided into several identical structural and functional units, the sarcomeres. The sarcomeres in turn are composed of the thin filaments which mainly consist of actin, tropomyosin and troponin, and the thick filaments which mainly consist of myosin.

20

25

The molecular mechanism of muscle contraction is based on a cyclic attachment and detachment of the globular myosin heads by the F actin filaments. On electrical stimulation of the myocardium, Ca^{2+} is released from the sarcoplasmic reticulum which influences, through an allosteric reaction, the troponin complex and tropomyosin, and thus opens the way for contact of the actin filament with the myosin

30

35

head. The attachment causes a conformational change in the myosin which thus pulls the actin filament along on itself. ATP is needed to reverse this conformational change and return to the start of a contraction cycle.

5 Short-term adjustment of the activity of the myocardium to the particular perfusion requirement, that is to say blood flow requirement, of the body is possible by nervous and hormonal regulation measures. It is thus possible to increase both the force of
10 contraction and the rate of contraction. Long-term overstrain results in physiological transformation processes in the myocardium, which are characterized mainly by an increase in myofibrils (myocyte hypertrophy).

15 If the myocardium is damaged, the originally physiological adaptation mechanisms often lead in the long term to pathophysiological states which develop into chronic cardiac insufficiency, that is to say cardiac weakness, and usually end with acute heart
20 failure. In cases of severe chronic insufficiency, the heart may no longer respond appropriately to changed output requirements, and even slight physical exertion leads to exhaustion and shortness of breath.

Damage to the myocardium results from
25 ischaemia, that is to say depletion of blood, caused by coronary disease, bacterial or viral infections, toxins, metabolic abnormalities, autoimmune diseases or genetic defects. Therapeutic measures are currently aimed at strengthening the force of contraction and
30 controlling the compensatory neuronal and hormonal compensation mechanisms. Despite this treatment, the mortality from this disease remains high (35-50% in the first 5 years after diagnosis). Cardiac insufficiency is the main cause of death in the world. The only
35 causal therapy is a heart transplant.

The molecular changes in chronic cardiac insufficiency are only inadequately known. In

particular, the genetic changes underlying cardiac insufficiency are substantially unknown. The question of why secondary damage by toxins or viruses leads to cardiac insufficiency in some people but not in others also remains unanswered.

The present invention is thus based on the object of identifying and isolating genes which are at least partly responsible for, if not in fact the causes of, genetically related cardiac disorders.

Surprisingly, a gene has now been found, in a human cardiac tissue cDNA bank, which is expressed more strongly in insufficient cardiac tissue than in healthy cardiac tissue and thus is causally connected with a genetically related cardiac insufficiency. A so-called EST (expressed sequence tag) already exists for this gene, although it is faulty and no function at all can be assigned to it (Tanaka, T. et al. (1996) Genomics, 35, 231-235; EMBL AC:CO4498; clone 3NHC3467).

One aspect of the invention is therefore a nucleic acid coding for a polypeptide having an amino acid sequence as shown in Fig. 4 or a functional variant thereof, and parts thereof having at least 8 nucleotides, preferably at least 10 nucleotides, in particular at least 15 nucleotides, especially at least 20 nucleotides, except a nucleic acid having the sequence:

```
1  GCCAACACGC ANTCCGACGA CAGTGCAGCC ATGGTCATTG CAGAGATGCN TCAAAGTCAA
61  TGAGCACATC ACCAACGTAA ACCTCGAGTC CAACTTCATA ACGGGAAAGG GGATCCTGGC
121 CATCATGAGA GCTCTCCAGC ACAACACGGT GCTCAGGAG CTGGGTTTCC ATAACCAGAG
181 GCACATCATG GGCAGCCAGG TGGAAATGGA GATTGTCAAG CTNCTGAAGG AGAACACGAC
241 GCTNCTGAGG CTGGGNTACC ATTTTNAACT CCCAGGACC
```

in which N denotes A, T, G or C.

The nucleic acid according to the invention was isolated from a human cardiac tissue cDNA bank and sequenced. For this, firstly complete RNA was isolated by standard methods from a healthy and insufficient cardiac sample and transcribed with the aid of a

3' anchor primer mixture, for example a 5'-T₁₂ACN-3' primer, in which N denotes any deoxyribonucleotide, and reverse transcriptase into c-DNA. The cDNA was then amplified with a method based on the so-called differential display method of Liang and Pardee (Liang, P. & Pardee, A. (1992) *Science* 257, 967-970) under specific PCR conditions with the aid of a 3' primer, for example a T₁₂ACN primer, and of an arbitrarily selected 5'-decamer primer, for example a 5'-CCTTCTACCC-3' decamer primer. It was possible thereby to amplify a 321 base pair (bp)-long DNA fragment which is surprisingly present not in the healthy heart sample but distinctly in the insufficient heart sample. This was surprising because the conventional methods such as the differential display method or else subtractive cDNA gene banks are associated with the problem of redundancy, of under-representation and of false-positive clones. In particular, it is possible to identify the gene products of weakly expressed genes only under special conditions. It is therefore also not astonishing that the hit rate is generally very low (10-20%) and, for example in the differential display method, also depends on the chosen PCR conditions, the primer length or, for example in the production of subtractive banks, on the hybridization temperature. The complete gene was then isolated from a cDNA gene bank with the aid of the found DNA fragment and sequenced.

In every case it is necessary to find out by further methods whether the found cDNA can be assigned to an active and/or tissue-specific gene. Hence mRNAs from various human tissues were hybridized with the found DNA fragment in a so-called Northern blot, and the amount of bound m-RNA was determined, for example, via the radiolabelling of the DNA fragment. This experiment led to detection of the corresponding RNA in particular in striped muscle, that is to say myocardial

and skeletal muscle tissue, and very weakly in prostate tissue. In a further experiment comparing between healthy and insufficient cardiac tissues, increased expression was detected, for example expression of the
5 RNAs increased by about 35%, in insufficient tissue by comparison with healthy tissue. It was possible to demonstrate in particular that a relatively small RNA species preferentially shows increased expression in insufficient tissue by comparison with healthy tissue.
10 The increased expression of the relatively small RNA species is readily evident for example in the Northern blot in the form of a double band (see Fig. 5b).

Comparison of the derived polypeptide sequence with a protein database additionally revealed a certain
15 relationship (homology) with the protein tropomodulin (see Fig. 4). Tropomodulin is known to be a polypeptide which in chicken cardiomyocytes has an influence on the development of the myofibrils and the contractility of the cells (Gregorio et al. (1995) *Nature* 377, 83-86).
20 This protein binds on the one hand to tropomyosin, and on the other hand to the actin filaments, but is not itself regulated in its activity. The derived polypeptide according to the invention likewise has some of the structural features of tropomodulins, such
25 as, for example, a tropomyosin binding domain. In contrast to tropomodulin, the polypeptide according to the invention has additional structural features indicating regulation of the activity of the polypeptide by so-called tyrosine kinases (see Fig. 4).

30 The term "functional variant" therefore means for the purpose of the present invention polypeptides which are functionally related to the polypeptide according to the invention, that is to say can likewise be referred to as a regulable modulator of the
35 contractility of myocardial cells, are expressed in striped muscle, preferably in myocardial, skeletal muscle and/or prostate tissue, especially in myocardial

and/or skeletal muscle and, in particular, in myocardial cells, have structural features of tropomodulin, such as, for example, one or more tropomyosin binding domains, and/or whose activity can
5 be regulated by tyrosine kinases. Examples of functional variants are the corresponding polypeptides derived from other organisms than humans, preferably from non-human mammals such as, for example, monkeys.

In the wider sense, the term „functional
10 variant“ includes polypeptides which have a sequence homology, in particular a sequence identity, of about 70%, preferably about 80%, in particular about 90%, especially about 95%, with the polypeptide having the amino acid sequence shown in Fig. 4. These include, for
15 example, polypeptides encoded by a nucleic acid which is isolated from non-heart-specific tissue, for example skeletal muscle tissue, but which has, after expression in a heart-specific cell, the identified function(s). These furthermore include deletions of the polypeptide
20 in the region of about 1-60, preferably of about 1-30, in particular of about 1-15, especially of about 1-5, amino acids. For example, the first amino acid methionine can be absent with negligible alteration in the function of the polypeptide. These also include
25 fusion proteins which comprise the above-described polypeptides according to the invention, it being possible for the fusion proteins themselves to have the function of a regulable modulator of the contractility of myocardial cells, or to acquire the specific
30 function only after elimination of the fusion portion. They particularly include fusion proteins with a content of, in particular, non-heart-specific sequences of about 1-200, preferably about 1-150, in particular about 1-100, especially about 1-50, amino acids.
35 Examples of non-heart-specific peptide sequences are prokaryotic peptide sequences which may be derived, for example, from the galactosidase of E. coli.

The nucleic acid according to the invention is generally a DNA or RNA, preferably a DNA. Preferred for expression of the relevant gene is in general a double-stranded DNA and for use as probe is a single-stranded
5 DNA. Particular preference is given to a double- or single-stranded DNA having a nucleic acid sequence as shown in Fig. 1, 2 or 3 and the parts thereof described in detail above, with the DNA region coding for the polypeptide being particularly preferred. This region
10 starts with the nucleic acids "ATG" coding for methionine at position 89 to "TAG" coding for "amber" (stop) at position 1747.

The nucleic acid according to the invention can, for example, be chemically synthesized on the
15 basis of the sequences disclosed in Figs. 1-3 or on the basis of the polypeptide sequence disclosed in Fig. 4 by use of the genetic code, for example by the phosphotriester method (see, for example, Uhlmann, E. & Peyman, A. (1990) *Chemical Reviews*, 90, 543-584,
20 No. 4). Another possibility for obtaining the nucleic acid according to the invention is isolation from a suitable gene bank, for example from a heart-specific gene bank, using a suitable probe (see, for example, J. Sambrook et al., (1989), *Molecular Cloning. A*
25 *Laboratory Manual* 2nd edn., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY). Suitable as probe are, for example, single-stranded DNA fragments with a length of about 100-1000 nucleotides, preferably with a length of about 200-500 nucleotides, in particular with
30 a length of about 300-400 nucleotides, whose sequence can be derived from the nucleic acid sequences shown in Figs. 1-3. One example of a probe is the DNA fragment of Example 1, which is 321 bp in size and corresponds to the underlined region in Fig. 1, using which the
35 nucleic acid according to the invention has already been isolated successfully from human cardiac tissue (see Example 2).

The nucleic acid according to the invention is normally present in a vector, preferably in an expression vector or vector effective for gene therapy. The vector effective for gene therapy preferably
5 contains heart-specific regulatory sequences such as, for example, the troponin C (cTNC) promoter (see, for example, Parmacek, M.S. et al. (1990) *J. Biol. Chem.* 265 (26) 15970-15976 and Parmacek, M.S. et al. (1992) *Mol. Cell Biol.* 12(5), 1967-1976), which is
10 functionally connected to the nucleic acid according to the invention.

The expression vectors may be prokaryotic or eukaryotic expression vectors. Examples of prokaryotic expression vectors for expression in *E. coli* are, for
15 example, the vectors pGEM or pUC derivatives, and of eukaryotic expression vectors for expression in *Saccharomyces cerevisiae* are, for example, the vectors p426Met25 or p426GAL1 (Mumberg et al. (1994) *Nucl. Acids Res.*, 22, 5767-5768) for expression in insect
20 cells are, for example, baculovirus vectors as disclosed in EP-B1 0 127 839 or EP-B1 0 549 721, and for expression in mammalian cells are, for example, the vectors Rc/CMV and Rc/RSV or SV40 vectors, which are all generally available.

The expression vectors generally also contain
25 promoters suitable for the particular host cell, such as, for example, the trp promoter for expression in *E. coli* (see, for example, EP-B1 0 154 133), the ADH2 promoter for expression in yeasts (Russell et al. (1983), *J. Biol. Chem.* 258, 2674-2682), the baculovirus polyhedrin promoter for expression in insect cells
30 (see, for example, EP-B1 0 127 839) or the SV40 early promoter or LTR promoters, for example of MMTV (mouse mammary tumour virus; Lee et al. (1981) *Nature* 214, 228-232).

Examples of vectors effective for gene therapy are virus vectors, preferably adenovirus vectors, in

particular replication-deficient adenovirus vectors, or adeno-associated virus vectors, for example an adeno-associated virus vector which consists exclusively of two inverted terminal repeats (ITR).

- 5 An adenovirus vector and, in particular, a replication-deficient adenovirus vector are particularly preferred for the following reasons.

 The human adenovirus belongs to the class of double-stranded DNA viruses with a genome of about 36
10 kilobase pairs (kb). The viral DNA codes for about 2700 different gene products, a distinction being made between early ("*early genes*") and late ("*late genes*"). The "*early genes*" are divided into four transcriptional units E1 to E4. The late gene products code for the
15 capsid proteins. It is possible to distinguish immunologically at least 42 different adenoviruses and subgroups A to F, all of which are suitable for the present invention. A precondition for transcription of the viral genes is expression of the E1 region which
20 codes for a transactivator of adenoviral gene expression. This dependence of the expression of all subsequent viral genes on the E1 transactivator can be utilized to construct adenoviral vectors not capable of replication (see, for example, McGrory, W.J. et al.
25 (1988) *Virology* 163, 614-617 and Gluzman, Y. et al. (1982) in "*Eukaryotic Viral Vectors*" (Gluzman, Y. ed.) 187 - 192, Cold Spring Harbor Press, Cold Spring Harbor, New York). In adenoviral vectors, especially of type 5 (for sequence, see Chroboczek, J. et al. (1992)
30 *Virology* 186, 280-285) and especially of subgroup C, in general the E1 gene region is replaced by a foreign gene with its own promoter or by the nucleic acid construct according to the invention. Replacement of the E1 gene region which is a precondition for
35 expression of the downstream adenoviral genes results in an adenovirus not capable of replication. These

viruses are then able to replicate only in a cell line which replaces the missing E1 genes.

Replication-deficient adenoviruses are therefore generally formed by homologous recombination in the so-called 293 cell line (human embryonic kidney cell line) which has a copy of the E1 region stably integrated into the genome. For this purpose, the nucleic acid according to the invention is cloned into recombinant adenoviral plasmids under the control of its own promoter, for example the troponin C promoter mentioned above. Homologous recombination then takes place with an E1-deficient adenoviral genome such as, for example, dl327 or del1324 (adenovirus 5) in the 293 helper cell line. Where recombination is successful, viral plaques are harvested. The replication-deficient viruses produced in this way are employed in high titres (for example 10^9 to 10^{11} plaque forming units) for infecting the cell culture or for somatic gene therapy.

The exact site of insertion of the nucleic acid according to the invention into the adenoviral genome is in general not critical. It is, for example, also possible to clone the nucleic acid according to the invention in place of the deleted E3 gene (Karlsson, S. et al. (1986), *EMBO J.* 5, 2377 - 2385).

However, it is preferred for the E1 region or parts thereof, for example the E1A or E1B region (see, for example, WO 95/00655), to be replaced by the nucleic acid according to the invention, especially when the E3 region is also deleted.

However, the present invention is not confined to the adenoviral vector system; on the contrary, adeno-associated virus vectors are also particularly suitable in combination with the nucleic acid according to the invention for the following reasons.

The AAV virus belongs to the family of parvoviruses. These are distinguished by an

icosahedral, non-enveloped capsid which has a diameter of 18 to 30 nm and which contains a linear, single-stranded DNA of about 5 kb. For efficient replication of AAV, coinfection of the host cell with helper viruses is necessary. Examples of suitable helpers are adenoviruses (Ad5 or Ad2), herpesviruses and vaccinia-viruses (Muzyczka, N. (1992) *Curr. Top. Microbiol. Immunol.* 158, 97-129). In the absence of a helper virus, AAV passes into a latency state where the virus genome is able to integrate stably into the host cell genome. The property of AAV integrating into the host genome makes it particularly interesting as transduction vector for mammalian cells. Generally sufficient for the vector functions are the two inverted terminal repeats (ITR: see, for example, WO 95/23867) which are about 145 bp long. They carry the signals necessary in "cis" for replication, packaging and integration into the host cell genome. For packaging into recombinant vector particles, a vector plasmid which harbours the genes for non-structural proteins (rep proteins) and for structural proteins (cap proteins) is transfected into adenovirus-infected cells. After a few days, a cell-free lysate containing, besides the recombinant AAV particles, also adenoviruses is prepared. The adenoviruses can advantageously be removed by heating at 56°C or by banding in a caesium chloride gradient. It is possible with this cotransfection method to achieve rAAV titres of 10^5 to 10^6 IE/ml. Contamination by wild-type viruses is below the detection limit if the packaging plasmid and the vector plasmid have no overlapping sequences (Samulski, R.J. (1989) *J. Virol.* 63, 3822 - 3828).

Transfer of the nucleic acid according to the invention into somatic cells can be effected by AAV into resting, differentiated cells, which is particularly advantageous for gene therapy of the heart. The ability to integrate which has been

mentioned also ensures long-lasting gene expression in vivo, which in turn is particularly advantageous. A further advantage of AAV is that the virus is not pathogenic for humans and is relatively stable in vivo.

5 Cloning of the nucleic acid according to the invention into the AAV vector or parts thereof takes place by methods known to the skilled person, as described, for example, in WO 95/23867, in Chiorini, J.A. et al. (1995), *Human Gene Therapy* 6, 1531-1541 or Kotin, R.M.

10 (1994), *Human Gene Therapy* 5, 793-801.

Vectors effective for gene therapy can also be obtained by complexing the nucleic acid according to the invention with liposomes, because it is possible thereby to achieve a very high transfection efficiency,

15 in particular of myocardial cells (Felgner, P.L. et al. (1987), *Proc. Natl. Acad. Sci. USA* 84, 7413-7417). In lipofection, small unilamellar vesicles of cationic lipids are prepared by ultrasound treatment of the liposome suspension. The DNA is bound ionically to the

20 surface of the liposomes, specifically in a ratio such that a positive net charge remains and the plasmid DNA is 100% complexed by the liposomes. Besides the lipid mixtures DOTMA (1,2-dioleoyloxypropyl-3-trimethylammonium bromide) and DOPE (dioleoylphosphatidyl-

25 ethanolamine) employed by Felgner et al. (1987, supra), numerous new lipid formulations have now been synthesized and tested for their efficiency in transfecting various cell lines (Behr, J.P. et al. (1989), *Proc. Natl. Acad. Sci. USA* 86, 6982-6986;

30 Felgner, J.H. et al. (1994) *J. Biol. Chem.* 269, 2550-2561; Gao, X. & Huang, L. (1991), *Biochim. Biophys. Acta* 1189, 195-203). Examples of the novel lipid formulations are DOTAP N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium ethyl

35 sulphate or DOGS (TRANSFECTAM; dioctadecylamidoglycylspermine). One example of the preparation of DNA-liposome complexes and successful use thereof in

heart-specific transfection is described in DE 44 11 402.

For use of the nucleic acid according to the invention in gene therapy, it is also advantageous if the part of the nucleic acid which codes for the polypeptide contains one or more noncoding sequences, including intron sequences, preferably between the promoter and the start codon of the polypeptide, and/or a polyA sequence, in particular the naturally occurring polyA sequence or an SV40 virus polyA sequence, especially at the 3' end of the gene, because this makes it possible to stabilize the mRNA in the myocardial cell (Jackson, R.J. (1993) *Cell* 74, 9-14 and Palmiter, R.D. et al. (1991) *Proc. Natl. Acad. Sci. USA* 88, 478-482).

The present invention further relates to the polypeptide itself having an amino acid sequence as shown in Fig. 4 or a functional variant thereof, and parts thereof having at least 6 amino acids, preferably having at least 12 amino acids, in particular having at least 15 amino acids and especially having at least 164 amino acids, except a polypeptide having the sequence:

PTRNPTTVQPWSLQRCIKVNEHITNVNVESNFITGKGILAIMRALQ

10	20	30	40
HNTVLTLELRFHNQRHIMGSQVEMEIVKLLKENTLLRLGYHFKLPG			
50	60	70	80
90			

The polypeptide is prepared, for example, by expression of the nucleic acid according to the invention in a suitable expression system as described above using methods generally known to the skilled person. Examples of suitable host cells are the *E. coli* strains DH5, HB101 or BL21, the yeast strain *Saccharomyces cerevisiae*, the lepidopteran insect cell line for example *Spodoptera frugiperda*, or the animal

cells COS, Vero, 293 and HeLa, all of which are generally obtainable.

The said parts of the polypeptide can also be synthesized by classical synthesis (Merrifield technique). They are particularly suitable for obtaining antisera which can be used to screen suitable gene expression banks in order thus to obtain further functional variants of the polypeptide according to the invention.

The present invention therefore relates also to antibodies which react specifically with the polypeptide having an amino acid sequence as shown in Fig. 4 or a functional variant thereof, and parts thereof having at least 6 amino acids, preferably having at least 12 amino acids, in particular having at least 15 amino acids and especially having at least 164 amino acids, the abovementioned parts of the polypeptide either themselves being immunogenic or being able to be made immunogenic, or have their immunogenicity increased, by coupling to suitable carriers such as, for example, bovine serum albumin.

The antibodies are either polyclonal or monoclonal. The preparation, to which the present invention also relates, takes place, for example, by generally known methods, by immunizing a mammal, for example a rabbit, with the said polypeptide or the said parts thereof, where appropriate in the presence of, for example, Freund's adjuvant and/or aluminium hydroxide gels (see, for example, Diamond, B.A. et al. (1981) *The New England Journal of Medicine*, 1344-1349). The polyclonal antibodies raised in the animal on the basis of an immunological response can then easily be isolated from the blood by generally known methods and purified, for example, by column chromatography. It was thus possible, for example, to produce in rabbits a polyclonal antiserum against a polypeptide which had amino acids 1-90 according to the invention, as shown

in Fig. 4, which was expressed as fusion protein in bacteria and purified by affinity chromatography. The antibodies according to the invention specifically recognized the corresponding protein of about 80 kD in extracts of human heart tissue.

Monoclonal antibodies can be prepared, for example, by the known method of Winter & Milstein (Winter, G. & Milstein, C. (1991) *Nature*, 349, 293-299).

The present invention also relates to a medicinal product which contains a nucleic acid coding for a polypeptide having an amino acid sequence as shown in Fig. 4 or a functional variant thereof and the abovementioned parts thereof having at least 8 nucleotides, or a polypeptide having an amino acid sequence as shown in Fig. 4 or a functional variant thereof and the abovementioned parts thereof having at least 6 amino acids and, where appropriate, suitable additives or excipients, and to a process for producing a medicinal product for treating cardiac disorders, in particular cardiac insufficiency, in which a said nucleic acid or a said polypeptide is formulated with a pharmaceutically acceptable carrier.

One example of the use of nucleic acid fragments as therapeutic agent is the use of DNA fragments in the form of antisense oligonucleotides (Uhlmann, E. & Peyman, A. (1990) *Chemical Reviews*, 90, 543-584, No. 4).

A particularly suitable medicinal product for use for human gene therapy is one which contains the said nucleic acid in naked form or in the form of one of the vectors effective for gene therapy which are described above, or in a form complexed with liposomes. The pharmaceutical carrier is, for example, a physiological buffer solution, preferably with a pH of about 6.0-8.0, preferably of about 6.8-7.8, in particular of about 7.4 and/or an osmolarity of about

200-400 milliosmol/litre, preferably of about 290-310 milliosmol/litre. The pharmaceutical carrier may additionally contain suitable stabilizers such as, for example, nuclease inhibitors, preferably complexing agents such as EDTA and/or other excipients known to the skilled person.

The said nucleic acid is normally administered intravenously, for example with the aid of a catheter, where appropriate in the form of the virus vectors described in detail above or as liposome complexes. It is advantageous, for example, to infuse the nucleic acid according to the invention directly into the patient's coronary arteries (so-called percutaneous coronary gene transfer, PCGT), in particular in the form of recombinant adenovirus vectors or adeno-associated virus vectors. Administration with the aid of a balloon catheter is particularly preferred because it is possible thereby to confine the transfection not only to the heart but also to the injection site within the heart (see, for example, Feldman, L.J. et al. (1994) JACC 235A, 906-934).

It is also possible to administer the polypeptide itself intravenously or with the aid of a catheter or balloon catheter, where appropriate with suitable additives or excipients, such as, for example, physiological saline, stabilizers, proteinase inhibitors etc., in order to influence the function of the heart immediately and directly.

The present invention further relates to a diagnostic aid containing a nucleic acid, a polypeptide or antibody according to the present invention and, where appropriate, suitable additives or excipients and to a process for producing a diagnostic aid for diagnosing cardiac disorders, in particular cardiac insufficiency, in which a nucleic acid, a polypeptide or antibody according to the present invention is mixed with suitable additives or excipients.

It is possible, for example, according to the present invention to produce on the basis of the said nucleic acid a diagnostic aid based on the polymerase chain reaction (PCR diagnosis, for example as disclosed in EP-0 200 362) or on a Northern blot as described in detail in Example 3 using the 321 bp DNA fragment according to the invention as probe. These tests are based on the specific hybridization of said nucleic acids with the complementary strand, normally of the corresponding mRNA. The nucleic acid may also in this case be modified as described, for example, in EP 0 063 879. A DNA fragment, in particular the DNA fragment described in Example 1, is preferably labelled using suitable reagents, for example radioactively with α -³²P-dCTP or non-radioactively with biotin, by generally known methods and incubated with isolated RNA, which has preferably been pre-bound to suitable membranes made of, for example, cellulose or nylon. It is additionally advantageous, before the hybridization and binding to a membrane, for the isolated RNA to be fractionated according to size, for example by agarose gel electrophoresis. With the same amount of investigated RNA from each tissue sample, it is thus possible to determine the amount of mRNA specifically labelled by the probe.

It is thus possible by using the diagnostic aid according to the invention also to measure a cardiac tissue sample in vitro specifically for the strength of expression of the corresponding gene in order to be able to diagnose reliably possible cardiac insufficiency (see Example 1). A cDNA having a sequence as shown in Fig. 1 is particularly suitable for diagnosing a possible cardiac insufficiency (see Example 2).

A further diagnostic aid contains the polypeptide according to the present invention or the immunogenic parts thereof described above in detail.

The polypeptide or the parts thereof, which are preferably bound to a solid phase, for example made of nitrocellulose or nylon, can, for example, be brought into contact in vitro with the body fluid to be investigated, for example blood, in order to react for example with autoimmune antibodies. The antibody-peptide complex can then be detected for example by means of labelled antihuman IgG or antihuman IgM antibodies. The label is, for example, an enzyme such as peroxidase which catalyses a colour reaction. The presence and the amount of autoimmune antibody present can thus be detected easily and rapidly by the colour reaction.

Another diagnostic aid contains the antibodies according to the invention themselves. These antibodies can be used, for example, for investigating a cardiac tissue sample easily and quickly to find whether the relevant polypeptide is present in an increased amount, in order thus to obtain information about possible cardiac insufficiency. In this case, the antibodies according to the invention are labelled for example with an enzyme, as already described above. The specific antibody-peptide complex can thus be detected easily and equally quickly by an enzymatic colour reaction.

The present invention also relates to a test for identifying functional interactors containing a nucleic acid according to the invention coding for a polypeptide having an amino acid sequence as shown in Fig. 4 or a functional variant thereof and the abovementioned parts thereof having at least 8 nucleotides, a polypeptide having the amino acid sequence as shown in Fig. 4 or a functional variant thereof, and the abovementioned parts thereof having at least 6 amino acids or the antibodies according to the invention and, where appropriate, suitable additives or excipients.

A suitable test for identifying functional interactors is, for example, the so-called two-hybrid system (Fields, S. & Sternglanz, R. (1994) *Trends in Genetics*, 10, 286-292).

5 In this test, a cell, for example a yeast cell, is transformed or transfected with one or more expression vectors which express a fusion protein which contains a polypeptide according to the present invention and a DNA binding domain of a known protein,
10 for example of Gal4 or LexA from *E. coli*, and/or expresses a fusion protein which contains an unknown polypeptide and a transcription activating domain, for example of Gal4, herpes virus VP16 or B42. The cell additionally contains a reporter gene, for example the
15 lacZ gene from *E. coli*, green fluorescence protein or the yeast amino acid biosynthesis genes His3 or Leu2, which is controlled by regulatory sequences, such as, for example, the LexA promoter/operator or by a so-called upstream activation sequence (UAS) of yeast. The
20 unknown polypeptide is encoded, for example, by a DNA fragment which is derived from a gene bank, for example from a human cardiac tissue-specific gene bank. Normally a cDNA gene bank is produced directly, using the expression vectors described, in yeast so that the
25 test can be carried out immediately thereafter.

For example, a nucleic acid according to the present invention is cloned into a yeast expression vector in a functional unit with the nucleic acid coding for the LexA DNA binding domain, so that a
30 fusion protein consisting of the polypeptide according to the invention and the LexA DNA binding domain is expressed in the transformed yeast. In another yeast expression vector, cDNA fragments from a cDNA gene bank are cloned in a functional unit with the nucleic acid
35 coding for the Gal4 transcription activating domain, so that a fusion protein consisting of an unknown polypeptide and the Gal4 transcription activating

domain is expressed in the transformed yeast. The yeast which is transformed with the two expression vectors and is, for example, Leu2⁻ additionally contains a nucleic acid which codes for Leu2, and is controlled by the LexA promoter/operator. In the event of a functional interaction between the polypeptide according to the invention and the unknown polypeptide, the Gal4 transcription activating domain binds via the LexA DNA binding domain to the LexA promoter/operator, whereby the latter is activated and the Leu2 gene is expressed. The result of this is that the Leu2⁻ yeast is able to grow on minimal medium which contains no leucine.

On use of the lacZ or green fluorescence protein reporter gene in place of an amino acid biosynthesis gene, activation of transcription can be detected by the formation of blue or green-fluorescing colonies. The blue or fluorescent coloration can also be quantified easily in a spectrophotometer, for example at 585 nm in the case of a blue coloration.

Thus, it is possible to screen expression gene banks easily and quickly for polypeptides which interact with a polypeptide according to the present invention. It is then possible for the novel peptides found to be isolated and further characterized.

Another possible use of the two-hybrid system is the influence on the interaction between a polypeptide according to the present invention and a known or unknown polypeptide by other substances such as, for example, chemical compounds. Thus, it is also possible to find easily novel and valuable active substances which can be chemically synthesized and can be employed as therapeutic agent for treating a cardiac disorder. The present invention is therefore not restricted to a method for finding polypeptide-like interactors, but also extends to a method for finding substances which are able to interact with the protein-

protein complex described above. Such polypeptide-like, as well as chemical interactors are therefore referred to as functional interactors for the purpose of the present invention.

5 The surprising advantage of the present invention is thus the possibility of using the subject-matters according to the invention for specific and reliable diagnosis and therapy of cardiac disorders, especially cardiac insufficiency. However, other
10 valuable therapeutic and diagnostic possibilities also emerge. For example, the functional interactors which can be easily identified using the described test methods are so advantageous because it is possible with their aid in the form of suitable medicinal products to
15 influence deliberately the activity of the polypeptide according to the invention in its natural environment in the myocardium and thus also the contractility of the myocardial cells, in particular since the activity of this polypeptide can be regulated as already
20 described in detail above.

 The following figures and examples are intended to illustrate the invention in detail without restricting it.

25 Description of the figures

 Fig. 1 shows a 1936 nucleotide-long heart-specific DNA sequence. The region which codes for the corresponding polypeptide is shown in bold. The DNA fragment from Example 1 is underlined.

30 Fig. 2 shows a 2080 nucleotide-long heart-specific DNA sequence which has an extension at the 5' end of the DNA sequence from Fig. 1. The region which codes for the corresponding polypeptide is once again shown in bold.

35 Fig. 3 shows a 2268 nucleotide-long heart-specific DNA sequence which has an extension at the 5' end of the DNA sequence from Fig. 1 or Fig. 2. The

region which codes for the corresponding polypeptide is likewise shown in bold.

Fig. 4 shows a 552 amino acid-long polypeptide sequence encoded by one of the DNA sequences shown in Figs. 1-3. The regions homologous with human tropomodulin are shown in bold. The sequence motifs which indicate regulation of the polypeptide by tyrosine kinase signal transduction pathways are underlined.

Figs. 5a and 5b show Northern blots of mRNAs which correspond to the nucleic acid sequences shown in Figs. 1-3 for detecting expression in various human tissues (Fig. 5a) and for detecting expression in healthy and insufficient human cardiac tissue (Fig. 5b).

Examples

1. Isolation of a DNA fragment from human insufficient cardiac tissue

Complete RNA was initially isolated by standard methods (Chomczynski & Sacchi (1987), *Anal. Biochem*, 162 (1), 156-159) from a healthy and an insufficient cardiac tissue sample. The RNA was then treated with DNase in order to remove DNA contamination. An aliquot of this RNA (0.2 μ g) was then incubated in a 20 μ l reaction mix with 1 \times RT buffer (Gibco Y00121), 10 mM DTT, 20 μ M dNTP mix, 1 U/ μ l RNasin (Promega N2511), 1 μ M 3' anchor primer mixture of the 5'-T₁₂ACN-3' type, where N can be any deoxynucleotide, and 10 U/ μ l SuperScript RNase H⁻ reverser transcriptase at 37°C for 60 min and thus transcribed into cDNA. A cDNA aliquot was then subjected to a 20 μ l PCR in 1 \times PCR buffer (Perkin-Elmer) which, besides 1 μ M 3' primer T₁₂AC and 1 μ M 5'-decamer primer (5'-CCTTCTACCC-3'), contains 10 μ Ci of α -³²P-dCTP, 2 μ M dNTP mix and 1 U of AmpliTaq (Perkin Elmer). The mixture was incubated firstly at 94°C for 1 min, then 40 cycles each of 30 s at 94°C,

40°C for 2 min and 72°C for 30 s and finally at 72°C for 10 min. The resulting DNA fragment mixture was then fractionated on a 6% polyacrylamide gel and autoradiographed. A DNA fragment which is 321 bp in length and which is not present in the healthy heart sample but is distinctly present in the insufficient heart sample is thus prepared. This fragment was then cut out of the gel on the basis of the X-ray film and was reamplified by PCR under the conditions already described. The resulting fragment was then cloned into an appropriate vector, and the DNA sequence was determined. A fragment prepared in this way contains nucleotides 1627-1936 of the sequence according to Claim 1 and the 12 thymine nucleotides from the 3' anchor primer.

2. Isolation of heart-specific nucleic acids

A plaque hybridization was carried with a cDNA gene bank from cardiac tissue under standard conditions (see Sambrook, J., Frisch, E.F. & Maniatis, T. (1989) *Molecular Cloning, A Laboratory Manual*, ch. 8-10) using an α -³²P-dCTP-labelled DNA fragment from Example 1 which comprises the nucleotides from position 1627-1936 in Fig. 1. The cDNAs found were then isolated and sequenced. The sequences are shown in Figs. 1-3. It emerged from this that the cDNA having the sequence shown in Fig. 1 could be isolated with greater probability from insufficient cardiac tissue than the cDNA having the sequence shown in Fig. 2 or 3, which could be isolated with greater probability from healthy cardiac tissue.

3. Detection of the strength of expression of the heart-specific gene in various human tissues by means of Northern blots.

The DNA fragment 321 bp in length already described in Examples 1 and 2 and Fig. 1 was firstly

radiolabelled with α -³²P-dCTP by the random primer labelling method (Feinberg, A.P. & Vogelstein, B. (1983) Anal. Biochem., 132, 6). The RTS RadPrime DNA labelling system (GibcoBRL 10387-017) was used for this purpose. The hybridization of blots with poly A⁺ RNA from human tissues (see Figs. 5a and 5b) took place at 68°C for 1 hour in accordance with the manufacturer's instructions (Multiple Tissue Northern Blots I & II, Clontech Laboratories GmbH, Heidelberg, #7760-1, #7759-1) in ExpressHyb hybridization solution (Clontech #8015-1). The blots were then washed with 2 × SSC and 0.05% SDS for 30 minutes and thereafter with 0.1 × SSC and 0.1% SDS for 1 hour and autoradiographed. It emerged that the probe 321 bp in length hybridizes strongly with a polyA⁺ RNA of about 2400 bp strongly in cardiac tissue and skeletal muscle, very weakly in prostate tissue and not in leucocytes, large intestinal, small intestinal, ovarian, testicular, thymus, splenic, renal, hepatic, lung, placental and brain tissue (Fig. 5a).

Expression of the corresponding RNAs in healthy and insufficient cardiac tissue was also investigated. Complete RNA was isolated from various human cardiac tissue samples for this purpose (Chomczynski & Sacchi (1987), Anal. Biochem. 162, 156-159). Subsequently in each case 10 µg of RNA were fractionated using a 1% formaldehyde agarose gel and transferred by the capillary method to a charged nylon membrane (Zeta-Probe GT BioRad #162-0197). The membrane was briefly washed with 2 × SSC and then baked at 80°C for 30 minutes. The membranes were incubated with prehybridization solution (0.5 M Na₂HPO₄, pH 7.2; 7% SDS) at 65°C for at least 1 hour. The solution was then replaced by a fresh solution, and the radioactive, heat-denatured probe was added. The hybridization was carried out at 65°C for 15 hours. The membranes were then washed firstly with 40 mM Na₂HPO₄, pH 7.2; 5% SDS

at 65°C for 15 hours and then with 40 mM Na₂HPO₄, pH 7.2; 1% SDS at 65°C for 2 x 30 minutes, and subsequently autoradiographed. It emerged that various RNA species having a length from about 2200 to 2400 bp were fractionated in 1% agarose gels. These different species correspond well with the sizes of the three cDNAs found, including an average polyA tail 150 bp long (see Figs. 1-3). In particular, the smallest RNA species was more clearly detectable in diseased tissue than in healthy tissue. Quantification of the blot using a PhosphorImager and the ImageQuant software (Molecular Dynamics GmbH, Krefeld), taking into account a control hybridization with β 4-thymosin and actin, revealed an approximately 35% increased expression of the detected RNAs in insufficient cardiac tissue by comparison with healthy tissue.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

5 (i) APPLICANT:

(A) NAME: MediGene Aktiengesellschaft

(B) STREET: Lochhamer Str. 11

(C) CITY: 82152 Martinsried

(D) COUNTRY: Germany

10 (F) POSTAL CODE: D-82152

(G) TELEPHONE: 089-89 56 32 0

(H) FAX: 089-89 56 32 00

(ii) TITLE OF INVENTION: Myocardium- and skeletal
15 muscle-specific nucleic acid, its
preparation and use

(iii) NUMBER OF SEQUENCES: 5

20 (iv) COMPUTER-READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: IBM PC compatible

(C) OPERATING SYSTEM: PC-DOS/MS-DOS

(D) SOFTWARE: Word Perfect 3.1

25

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1936 base pairs

30 (B) TYPE: nucleotide

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

35

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: YES
(vi) ORIGINAL SOURCE:
(F) TISSUE TYPE: cardiac tissue

5 (xi) SEQUENCE DESCRIPTION SEQ ID NO: 1:

CAGCGTGC	CTTGCTCCC	TGCTGCTTC	TGGGTGCTT	GAATGCTTG	TCCTTCAAG	81
TCCTTCTGG	TCTGACAA	CAGGACCAT	GTCTACCTT	GGCTACCGA	GAGGACTCAG	121
TAAATACGA	TCCATCGAG	AGGATGAAT	CCTCGCTCC	CTGTCAGCC	AGGAGCTGAA	181
GGAGCTAG	AGAGAGTTG	AAGACATTG	ACCTGACCG	AACCTTCCC	TGGGCTTAAG	241
GCAAAAGAG	CTGACAGAG	AAACCCCAT	AGGGACATT	AGCAGAGAG	CACTGATGG	301
CTATTGGGA	AAGGAGTCC	AAAACTCTT	GGAGAAGAG	AGGCTGGGG	AATGTGGAA	361
GGTTGCAGA	GACAAAGAG	AAAGTGAAG	AGAGCTTAT	TTTACTGAA	GTAAACAGT	421
GGTTTCTAG	GAAGTGTAT	CAGAGGAGG	GGAGGAGAG	TCCCAGGAG	AAGAGGAGG	481
AGAAGACAT	GACGAAGAG	AAAGAACAAT	TGAACATGC	AAAGGAGTA	ATGGAACGT	541
AAATTATGAT	AGTGTCAAT	CTGACAATC	TAAGCCAAAG	ATATTTAAA	GTCAAAATG	600
GAACATAAT	TTGACCAAT	GCAGCAATG	GAGGACACA	GAGTCCCAG	CTGCCATTCA	660
CCCTTGTGA	AATCTACAG	TGATTGAGG	CGCTTTGGAC	AAGATTAATA	GCAATGACCC	720
TGACACCACA	GAAGTCAAT	TGAACAACAT	TGAACAACAT	ACAACACAG	CCCTTACCG	780
CTTTGCTGA	GCCCTCAAG	ACAACACTGT	GGTGAAGAG	TTCACTCTG	CCAACACGG	840
TGCCGAGAC	AGTGACGCC	TGGCCATTGC	AGACATGCT	AAAGCCAATG	AGCACATCAC	900
CAACGTAAAC	GTGAGTCCA	ACTTCATAAC	GGGAAAGGG	ATCCTGGCCA	TCATGAGAG	960
TCTCCAGCAC	AACACGGTGC	TCACGGAGCT	GGTTTTCAT	AACACAGAG	ACATCATGG	1020
CAGCCAGGTG	GAAATGGAG	TTGTCAAGCT	GCTGAAGAG	AACACGACG	TGCTGAGGT	1080
GGGATACCAT	TTTGAATCC	CAGGACCAAG	AATGAGCATG	ACGAGCATTT	TGACAAGAAA	1140
TATGGATAAA	CAGAGGCAAA	AACGTTTGCA	GGAGCAAAAA	CAGCAGGAG	GATACGATG	1200
AGGACCCAAT	CTTAGGACCA	AAGTCTGGCA	AAGAGGAACA	CCTAGCTCTT	CACCTTATGT	1260
ATCTCCACAG	CACTCACCCT	GGTCTCCCC	AAAACTCCCC	AAAAAGTCC	AGACTGTGAG	1320
GAGCGTCTCT	CTGTCTCTG	TGGCCACACT	TCTCTCTCT	CCCCCTCTCT	CTCTCTCTCT	1380
CCCTCTCTCT	TCCCAAGGC	TGCCACACCC	TCTCTCTCT	CCCCCTCTCT	CACTCCACAG	1440
GAATAAGCTC	ATTACAGAA	ACATTGCAGA	AGTCATCAA	CAACAGGAGA	GTGCCCAACG	1500
GGCATTACAA	AATGGACAAA	AAAAAGAAAA	AGGGAAAAAG	GTCAAGAAAC	AGCCAAACAG	1560
TATTCTAAAG	GAATTAATAA	ATTCTCTAG	GTCAAGTCAA	GAGAAGAAAA	TGGAAGACAG	1620
TCTCCGACCT	TCTACCCAC	AGAGATCAGC	TCATGAGAA	CTCATGGAG	CAATTGGGG	1680
AAGCAGCATA	AAACAGCTAA	AGCGGGTGA	AGTTCCAGAA	GCCTTGGAT	GGGAACATGA	1740
TCTTTAGAG	AGGATGCAGA	ACTGTTCAGT	GGTATTACAT	GAATGCATT	GTGAGATGTT	1800
TCTAAATAAC	CTTCTTCAAT	TCAAAATGAT	CCCTGACTTT	AAAAATATC	TCACCCATTA	1860
ATTCCAAGA	GAATCTTAAG	AAACAATCAG	CATGTTTCTT	CTGTAAATAT	GAATAATAAT	1920
TCTTTTATA	TGTCGT					1986

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 2080 base pairs
(B) TYPE: nucleotide
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: YES

15 (vi) ORIGINAL SOURCE:

(F) TISSUE TYPE: cardiac tissue

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 2:

CAGCCTGCCA	CTTGCCTCCC	TGCCTGCTTC	TGGCTGCCIT	GAATGCCTGG	TCCTTCAAGC	50
TCCTTCTGGG	TCTGCACAAAG	CAGGGACCAT	GTCTACCTTT	GGCTACCGAA	GAGGACTCAG	120
TAATATCGAA	TCCATCGACG	AGGATGAAC	CCTCGCCTCC	CTGTACGCG	AGGAGCTGAA	180
GGAGCTAGAG	AGAGAGTTGG	AAGACATTGA	ACCTGACCGC	AACCTTCCCG	TGGGGCTAAG	240
GC AAAAGAGC	CTGACAGAGA	AAACCCCCAC	AGGGACATTC	AGCAGAGAGG	CACTGATGGC	300
CTATTGGGAA	AAGGAGTCCC	AAAAACTCTT	GGAGAAGGAG	AGGCTGGGGG	AATGTGGAAA	360
GGTTGCAGAA	GACAAAGAGG	AAAGTGAAGA	AGAGCTTATC	TTTACTGAAA	GTAACAGTGA	420
GGTTTCTGAG	GAAGTGTATA	CAGAGGAGGA	GGAGGAGGAG	TCCAGGAGG	AAGAGGAGGA	480
AGAAGACAGT	GACGAAGAGG	AAAGAACAA	TGAAACTGCA	AAAGGGATTA	ATGGAACTGT	540
AAATTATGAT	AGTGTCAATT	CTGACAACTC	TAAGCCAAAG	ATATTTAAAA	GTCAAATAGA	600
GAACATAAAT	TTGACCAATG	GCGACAAATG	GAGGAACACA	GAGTCCCCAG	CTGCCATTCA	660
CCCTTGTGGA	AATCTACAG	TGATTGAGGA	CGCTTTGGAC	AAGATTAAAA	GCAATGACCC	720
TGACACCACA	GAACTCAATT	TGAACAACAT	TGAGAACATC	ACACACAGA	CCCTTACCCG	780
CTTTGCTGAA	GGCTCAAAG	ACAACACTGT	GGTGAAGAGC	TTTACCTTGG	CCAACAGCA	840
TGCCGACGAC	AGTGCAGCTA	TGGCCATTGC	AGAGATGCTC	AAAGCCAAAT	AGCACATCAC	900
CAAGCTAAAC	GTGAGTCCA	ACTTCATAAC	GGGAAAAGGG	ATCCTGGCCA	TCATGAGAGC	960
TCTCCAGCAC	AACACGGTGC	TCACGGAGCT	CGGTTTCCAT	AACGAGAGGC	ACATCATGGG	1020
CAGCCAGGTG	GAATGGAGA	TTGTCAAGCT	GCTGAAGGAG	AACACGAGCC	TGCTGAGGCT	1080
GGGTATCAT	TTTGAATCCC	CAGGACCAAG	AATGAGATG	ACGAGCATTT	TGACAAAGAA	1140
TATGATATA	CAGAGGCAAA	AAGCTTTGCA	GGAGCAAAAA	CAGCAGAGGG	GATACGATGG	1200
AGGCCCAAT	CTTAGGACCA	AAGTCTGGCA	AAGAGGAAAC	CCTAGCTCTT	CACCTTATGT	1260
ATCTCCAGG	CACCTACCTT	GGTCATCCCC	AAAACTCCCC	AAAAAGATCC	AGACTGTGAG	1320
GAGCCCTCCT	GTGCTCCTG	TGGCCACACT	TCTCTCTCCT	CCCTCTCCTC	CTCCTCTCTC	1380
CCCTCTCTCT	TGCCAAAGGC	TGCCACCACT	TCTCTCTCCT	CCCTCTCTCT	CACCTCCAGA	1440
GA AAAAGCTC	ATTACCGAA	ACATTGAGA	AGTCATCAA	CAACAGGAGA	GTGCCCAACG	1500
GGCATTACAA	AATGGACAAA	AAAAAGAAAA	AGGGAAAAAG	GTCAAGAAAC	AGCCAAACAG	1560
TATTCTAAAG	GAATAAAAA	ATTCTCTGAG	GTCAAGTCAA	GAGAAGAAAA	TGGAAGACAG	1620
TTCCCGACCT	TCTACCCAC	AGAGATCAGC	TCATGAGAT	CTCATGGAAG	CAATTCCGGG	1680
AAGCAGCATA	AAACAGCTAA	AGCGGGTGGG	AGTTCCAGAA	CGCTTGCAT	GGGAACATGA	1740
TC TTTAAGAG	AGGATGCAGA	ACTGTTCAAT	GGTATTACAT	GAAATGCATT	GTGAGATGTT	1800
TC TAAAAATAC	CTTCTTCAAT	TCAAAATGAT	CCCTGACTTT	AAAAATAATC	TCACCCATTA	1860
ATTCCAAAGA	GAATCTTAA	AAACAATCAG	CATGTTCTTT	CTGTAAATAT	GAAAAATAAT	1920
TTCTTTTATA	TGTCGTGAGA	TTTGATTGG	CAAGAAGCAG	TTAATTTAAA	GATGCTCTTC	1980
CTATCTGTGG	ATGTGTTGGT	AACCTCGAGT	TGTAATGAGT	TCATGAATG	TGCTGTTATT	2040
TTGTATACT	CAATAAATGT	GGATTGAAGT	TTTTTCCCTT			2100

(2) INFORMATION FOR SEQ ID NO: 3:

5

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2268 base pairs

(B) TYPE: nucleotide
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

5 (ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: YES

10

(vi) ORIGINAL SOURCE:

(F) TISSUE TYPE: cardiac tissue

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 3:

15

CAGCGTGCCA	CTTGCGCTCCC	TGCGTGCTTC	TGGGTGCGCTT	GAATGCGCTGG	TCCTTCAAGC	60
TCCTTCTGGG	TCTGACAAAG	CAGGGACCAT	GTCTACCTTT	GGCTACCGAA	GAGGACTCAG	120
TAAATACGAA	TCGATCGACG	AGGATGAAC	CCTCGCCTCC	CTGTGAGCCG	AGGAGCTGAA	180
GGAGCTAGAG	AGAGAGTTGG	AAGACATTGA	ACCTGACCGC	AACCTTCCCG	TGGGGCTAAG	240
GTAAGAGAG	TTGACAGAGA	AAACCCCCAC	AGGACACATC	AGCAGAGAGG	CACTGATGGC	300
CTATTGGGAA	TAGAGTCCC	AAAACTCTT	GGAGAAGGAG	AGGCTGGGGG	AATGTGAAAA	360
GTTTTCGAA	TACAAAGAGG	AAAGTGAAGA	AGAGCTTATC	TTTACTGAAA	GTAAACGTGA	420
GTTTCTGAG	AAATGTGTATA	CAGAGGAGGA	GGAGGAGGAG	TCCAGGAGG	AAGAGGAGGA	480
ATAAGACAGT	TACAGAGAGG	AAAGAACAA	TGAAACTGCA	AAAGGGATTA	ATGGAAGTGT	540
AAATTATGAT	AGTGTCAATT	CTGACAAC	TAGCCAAAG	ATATTAAAA	GTCAAAATAGA	600
GAACATAAAT	TTGACCAATG	GCAGCAATGG	GAGGAACACA	GAGTCCCCAG	CTGCCATTCA	660
CCCTTGTGGA	AATCTACAG	TGATTGAGGA	CGCTTTGGAC	AAGATTAAAA	GCAATGACCC	720
TGACACCACA	GAAGTCAATT	TGAACAACAT	TGAGAACATC	ACAACACAGA	CCCTTACCCG	780
CTTTGCTGAA	CCCTTCAAGG	ACAACACTGT	GOTGAAGAGG	TTCAGTCTGG	CCAACACGCA	840
TGCCGACGAC	AGTGACGCCA	TGGCCATTGC	AGAGATGCTC	AAAGCCAATG	AGCACATCAC	900
CAACOTAAAC	GTGAGTCCA	ACTTCATAAC	GGGAAAGGGG	ATCCTGCGCA	TCATGAGAGC	960
TCTCCAGCAC	AACACGGTGC	TCACGGAGCT	CGCTTTCCAT	AACCAGAGGC	ACATCATGGG	1020
CAGCCAGGTG	GAATGTGAGA	TTGTCAAGCT	GCTGAAGGAG	AACACGAGCC	TGCTGAGGCT	1080
GGGATACCAAT	TTTGAACCTC	CAGGACCAAG	AATGAGCATG	ACGAGCATTT	TGACAAAGAAA	1140
TATGGATAAA	CAGAGGCAAA	AACGTTTGCA	GGAGCAAAAA	CAGCAGGAGG	GATACGATGG	1200
AGGACCCCAAT	CTTAGGACCA	AAGTCTGGCA	AAGAGGAACA	CCTAGCTCTT	CACCTTATGT	1260
ATCTCCGAGG	CACCTACCCCT	GCTCATCCCC	AAAACTCCCC	AAAAAAGTCC	AGACTGTGAG	1320
GAGCGCTCT	CTGTCTCTCT	TGGCCAGACT	TCCTCTCTCT	CCCCCTCTCT	CTCTCTCTCT	1380

CCCTCCTTCT	TCCCAAAGGC	TGCCACCACC	TCCTCCTCCT	CCCCCTCCTC	CACTCCCAGA	144C
GAAGAAAGCTC	ATTACCAGAA	ACATTCGAGA	AGTCATCAAA	CAACAGGAGA	GTGCCCAAGC	150C
GGCATTACAA	AATGGACAAA	AAAAGAAAAA	AGGGAAAAAG	GTCAAGAAAC	AGCCAAACAG	156C
TATTCTAAG	GAATAAAAA	ATTCTCTGAG	GTCAAGTCAA	GAGAAGAAAA	TGGAAGACAG	162C
TTCCCGACCT	TCTACCCAC	AGAGATCAGC	TCATGAGAA	CTCATGGAAG	CAATTCGGGG	168C
AAGCAGCATA	AAACAGCTAA	AGCGGGTGA	AGTCCAGAA	GCCCTGCGAT	GGGAACATGA	174C
TCITTAGAAG	AGGATGCAGA	ACTGTTCACT	GGTATTACAT	GAAATGCATT	GTGAGATGTT	180C
TCTAAATAC	CTTCTCAAT	TCAAAATGAT	CCCTGACTTT	AAAAATAATC	TCACCCATTA	186C
ATTCCAAAGA	GAATCTTAAG	AAACAATCAG	CATGTTTCTT	CTGTAATAT	GAAAATAAAT	192C
TTCTTTTTTA	TGTCGTGAGA	TTTGTAATGG	CAAGAAGCAG	TTAATTTAAA	GATGCTCTTC	198C
CTATCTGTGG	ATGTGTTGGT	AACCTCCAGT	TGTAATGAGT	TCATGAAATG	TGCTGTTATT	204C
TTTGTAATCT	CAATAAATGT	GGATTGAAGT	TTTTTCCCTT	TTTTTAAAGC	CAAACTAATA	210C
TTTTTCGTGG	ACTTGATACA	TCTGTCAGAT	TTTTGTAATC	TCGATAAATG	TGTATTGAAG	216C
TTTTTCCCT	TTTTTAAAA	AGCCAAACTA	ATATTTTCTC	GTGAGTTAAT	ACATCTGTCA	222C
GGTGTGTATG	TAACATTACT	GGACATTAAA	AAAAAATTAT	ACATTCTC		228C

(2) INFORMATION FOR SEQ ID NO: 4:

- 5 (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 552 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- 10 (ii) MOLECULE TYPE: protein
- (iii) HYPOTHETICAL: NO
- 15 (iv) ANTISENSE: YES
- (vi) ORIGINAL SOURCE:
- (F) TISSUE TYPE: cardiac tissue
- 20 (xi) SEQUENCE DESCRIPTION SEQ ID NO: 4:

Met	Ser	Thr	Phe	Gly	Tyr	Arg	Arg	Gly	Leu	Ser	Lys	Tyr	Glu	Ser	Ile
1				5					10					15	
Asp	Glu	Asp	Glu	Leu	Leu	Ala	Ser	Leu	Ser	Ala	Glu	Glu	Leu	Lys	Glu
		20						25					30		
Leu	Glu	Arg	Glu	Leu	Glu	Asp	Ile	Glu	Pro	Asp	Arg	Asn	Leu	Pro	Val
		35					40					45			
Gly	Leu	Arg	Gln	Lys	Ser	Leu	Thr	Glu	Lys	Thr	Pro	Thr	Gly	Thr	Phe
	50					55					60				

Ser Arg Glu Ala Leu Met Ala Tyr Trp Glu Lys Glu Ser Gln Lys Leu
65 70 75 80

Leu Glu Lys Glu Arg Leu Gly Glu Cys Gly Lys Val Ala Glu Asp Lys
85 90 95

Glu Glu Ser Glu Glu Glu Leu Ile Phe Thr Glu Ser Asn Ser Glu Val
100 105 110

Ser Glu Glu Val Tyr Thr Glu Glu Glu Glu Glu Ser Gln Glu Glu
115 120 125

Glu Glu Glu Glu Asp Ser Asp Glu Glu Glu Arg Thr Ile Glu Thr Ala
130 135 140

Lys Gly Ile Asn Gly Thr Val Asn Tyr Asp Ser Val Asn Ser Asp Asn
145 150 155 160

Ser Lys Pro Lys Ile Phe Lys Ser Gln Ile Glu Asn Ile Asn Leu Thr
165 170 175

Asn Gly Ser Asn Gly Arg Asn Thr Glu Ser Pro Ala Ala Ile His Pro
180 185 190

Cys Gly Asn Pro Thr Val Ile Glu Asp Ala Leu Asp Lys Ile Lys Ser
195 200 205

Asn Asp Pro Asp Thr Thr Glu Val Asn Leu Asn Asn Ile Glu Asn Ile
210 215 220

Thr Thr Gln Thr Leu Thr Arg Phe Ala Glu Ala Leu Lys Asp Asn Thr
225 230 235 240

Val Val Lys Thr Phe Ser Leu Ala Asn Thr His Ala Asp Ser Ala
245 250 255

Ala Met Ala Ile Ala Glu Met Leu Lys Ala Asn Glu His Ile Thr Asn
260 265 270

Val Asn Val Glu Ser Asn Phe Ile Thr Gly Lys Gly Ile Leu Ala Ile
275 280 285

Met Arg Ala Leu Gln His Asn Thr Val Leu Thr Glu Leu Arg Phe His
290 295 300

Asn Gln Arg His Ile Met Gly Ser Gln Val Glu Met Glu Ile Val Lys
305 310 315 320

Leu Leu Lys Glu Asn Thr Thr Leu Leu Arg Leu Gly Tyr His Phe Glu
325 330 335

Leu Pro Gly Pro Arg Met Ser Met Thr Ser Ile Leu Thr Arg Asn Met
340 345 350

Asp Lys Gln Arg Gln Lys Arg Leu Gln Glu Gln Lys Gln Gln Glu Gly
355 360 365

Tyr Asp Gly Gly Pro Asn Leu Arg Thr Lys Val Trp Gln Arg Gly Thr
370 375 380

Pro Ser Ser Ser Pro Tyr Val Ser Pro Arg His Ser Pro Trp Ser Ser
385 390 395 400

Pro Lys Leu Pro Lys Lys Val Gln Thr Val Arg Ser Arg Pro Leu Ser
405 410 415

Pro Val Ala Thr Leu Pro Pro Pro Pro Pro Pro Pro Pro Pro Pro
420 425 430

Pro Ser Ser Gln Arg Leu Pro Pro Pro Pro Pro Pro Pro Pro Pro
435 440 445

Leu Pro Glu Lys Lys Leu Ile Thr Arg Asn Ile Ala Glu Val Ile Lys
450 455 460

Gln Gln Glu Ser Ala Gln Arg Ala Leu Gln Asn Gly Gln Lys Lys Lys
465 470 475 480

Lys Gly Lys Lys Val Lys Lys Gln Pro Asn Ser Ile Leu Lys Glu Ile
485 490 495

Lys Asn Ser Leu Arg Ser Val Gln Glu Lys Lys Met Glu Asp Ser Ser
500 505 510

Arg Pro Ser Thr Pro Gln Arg Ser Ala His Glu Asn Leu Met Glu Ala
515 520 525

Ile Arg Gly Ser Ser Ile Lys Gln Leu Lys Arg Val Glu Val Pro Glu
530 535 540

Ala Leu Arg Trp Glu His Asp Leu
545 550

(2) INFORMATION FOR SEQ ID NO: 5:

- 5 (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 10 base pairs
 - (B) TYPE: nucleotide
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- 10 (ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: YES

5 (vi) ORIGINAL SOURCE:

(F) TISSUE TYPE: cardiac tissue

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 5

CCTTCTACCC

1:

10

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 279 base pairs

15

(B) TYPE: nucleotide

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

20

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: YES

25

(vi) ORIGINAL SOURCE:

(F) TISSUE TYPE: cardiac tissue

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 6:

GCCAACACGC	ANTCCGACGA	CAGTGCAGCC	ATGGTCATTG	CAGAGATGCN	TCAAAGTCAA	50
TGAGCACATC	ACCAACGTAA	ACGTCGAGTC	CAACTTCATA	ACGGGAAAGG	GGATCCTGGC	120
CATCATGAGA	GCTCTCCAGC	ACAACACGGT	GCTCAGCGAG	CTGGGTTTC	ATRACCCAGG	180
GCACATCATG	GGCAGCCAGG	TGGAAATGGA	GATTGTCAAG	CTNCTGAGG	AGAACACGAC	240
GCNCTGAGG	CTGGGTACC	ATTTTNAACT	CCGAGGACC			279

30

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

35

(A) LENGTH: 93 amino acids

(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

5 (ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: YES

10

(vi) ORIGINAL SOURCE:

(F) TISSUE TYPE: cardiac tissue

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 7:

15

PTRNPTTVQPWSLQRCIKVNEHITNVNVESNFITGKGILAIMRALQ
10 20 30 40
HNIVLTELRFHNQRHIMGSQVEMEIVKLLKENTLLRLGVHFKLPG
50 60 70 80 90

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M25519PC B0

Claims

5

1. Nucleic acid coding for a polypeptide having an amino acid sequence as shown in Fig. 4 or a functional variant thereof, and parts thereof having at least 8 nucleotides, except a nucleic acid having the sequence:

10

```
1  GCCAACACGC ANTCGACGA CAGTGCAGCC ATGGTCATTG CAGAGATGCN TCAAAATCAA
61  TGAGGCATC ACCAACGTAA ACGTGGAGTC CAACTTCATA ACGGGAAGG GGATCTTTC
121  CATCATGAGA GCTCTCCAGC ACAACACGGT GCTCAGGAG CTGCGTTTCC ATAACDAGAG
181  GCACATCATG GGCAGCCAGG TGGAAATGGA GATTGTCAAG CTNCTGAAGG AGAACATAC
241  GCTNCTGAGG CTGGNTACC ATTTTNACT CCCAGGACC
```

15

2. Nucleic acid according to Claim 1, characterized in that the nucleic acid is a DNA or RNA, preferably a DNA, in particular a double-stranded DNA.

3. Nucleic acid according to Claim 1 or 2, characterized in that the nucleic acid contains a DNA having a nucleic acid sequence as shown in Fig. 1, 2 or 3.

20

4. Nucleic acid according to any of Claims 1-3, characterized in that the nucleic acid is present in a vector, preferably in an expression vector or vector effective for gene therapy.

25

5. Nucleic acid according to any of Claims 1-4, characterized in that the part of the nucleic acid which codes for the polypeptide contains one or more noncoding sequences and/or a polyA sequence.

30

6. Process for the preparation of a nucleic acid according to any of Claims 1-5, characterized in that the nucleic acid is chemically synthesized or isolated from a gene bank using a probe.

7. Polypeptide having an amino acid sequence as shown in Fig. 4 or a functional variant thereof, and

parts thereof having at least 6 amino acids, except a polypeptide having the sequence:

PTRNPTTVQPWSLQRCIKVNEHITNVNVESNFTGKGILAIMRALQ

10

20

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40

HNTVLTELRFHNQRHIMGSQVEMEIVKLLKENTTLRLGYHFKLPG

50

60

70

80

90

5 8. Process for the preparation of a polypeptide according to Claim 7, characterized in that a nucleic acid according to any of Claims 1-3 is expressed in a suitable host cell.

9. Antibody against a polypeptide having an amino acid sequence as shown in Fig. 4 or a functional variant thereof, and parts thereof having at least 6 amino acids.

10 10. Process for the preparation of an antibody according to Claim 9, characterized in that a mammal is immunized with a polypeptide having an amino acid sequence as shown in Fig. 4 or a functional variant thereof, and parts thereof having at least 6 amino acids, and the resulting antibodies are isolated.

11. Medicinal product containing a nucleic acid coding for a polypeptide having an amino acid sequence as shown in Fig. 4 or a functional variant thereof, and parts thereof having at least 8 nucleotides, or a polypeptide having an amino acid sequence as shown in Fig. 4 or a functional variant thereof, and parts thereof having at least 6 amino acids, and, where appropriate, a pharmaceutically acceptable carrier.

12. Process for the preparation of a medicinal product for treating cardiac disorders, characterized in that a nucleic acid coding for a polypeptide having an amino acid sequence as shown in Fig. 4 or a functional variant thereof, and parts thereof having at least 8 nucleotides, or a polypeptide having an amino acid sequence as shown in Fig. 4 or a functional

variant thereof, and parts thereof having at least 6 amino acids, is formulated with a pharmaceutically acceptable carrier.

13. Diagnostic aid containing a nucleic acid coding
5 for a polypeptide having an amino acid sequence as shown in Fig. 4 or a functional variant thereof, and parts thereof having at least 8 nucleotides, a polypeptide having an amino acid sequence as shown in Fig. 4 or a functional variant thereof, and parts
10 thereof having at least 6 amino acids, or an antibody according to Claim 9 and, where appropriate, suitable additives or excipients.

14. Process for the preparation of a diagnostic aid for diagnosing cardiac disorders, characterized in that
15 a nucleic acid coding for a polypeptide having an amino acid sequence as shown in Fig. 4 or a functional variant thereof, and parts thereof having at least 8 nucleotides, a polypeptide having an amino acid sequence as shown in Fig. 4 or a functional variant
20 thereof, and parts thereof having at least 6 amino acids, or an antibody according to Claim 9, is mixed with a pharmaceutically acceptable carrier.

15. Test for identifying functional interactors containing a nucleic acid coding for a polypeptide
25 having an amino acid sequence as shown in Fig. 4 or a functional variant thereof, and parts thereof having at least 8 nucleotides, or a polypeptide having an amino acid sequence as shown in Fig. 4 or a functional variant thereof, and parts thereof having at least 6
30 amino acids, and, where appropriate, suitable additives or excipients.

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M25519PC BÖ

Abstract

The invention relates to a myocardium- and skeletal muscle-specific nucleic acid, its preparation and use as diagnostic aid, medicinal product and test for identifying functional interactors.

Fig. 1

CAGCCTGCCA	CTTGCCTCCC	TGCCTGCTTC	TGGCTGCCTT
GAATGCCTGG	TCCTTCAAGC	TCCTTCTGGG	TCTGACAAAG
CAGGGACCAT	GTCTACCTTT	GGCTACCGAA	GAGGACTCAG
TAAATACGAA	TCCATCGACG	AGGATGAACT	CCTCGCCTCC
CTGTCAGCCG	AGGAGCTGAA	GGAGCTAGAG	AGAGAGTTGG
AAGACATTGA	ACCTGACCGC	AACCTTCCCG	TGGGGCTAAG
GCAAAAGAGC	CTGACAGAGA	AAACCCCCAC	AGGGACATTC
AGCAGAGAGG	CACTGATGGC	CTATTGGGAA	AAGGAGTCCC
AAAAACTCTT	GGAGAAGGAG	AGGCTGGGGG	AATGTGGAAA
GGTTGCAGAA	GACAAAGAGG	AAAGTGAAGA	AGAGCTTATC
TTTACTGAAA	GTAACAGTGA	GGTTTCTGAG	GAAGTGTATA
CAGAGGAGGA	GGAGGAGGAG	TCCCAGGAGG	AAGAGGAGGA
AGAAGACAGT	GACGAAGAGG	AAAGAACAAT	TGAAACTGCA
AAAGGGATTA	ATGGAACGTG	AAATTATGAT	AGTGTCAATT
CTGACAACTC	TAAGCCAAAG	ATATTTAAAA	GTCAAATAGA
GAACATAAAT	TTGACCAATG	GCAGCAATGG	GAGGAACACA
GAGTCCCCAG	CTGCCATTCA	CCCTTGTGGA	AATCCTACAG
TGATTGAGGA	CGCTTTGGAC	AAGATTAAAA	GCAATGACCC
TGACACCACA	GAAGTCAATT	TGAACAACAT	TGAGAACATC
ACAACACAGA	CCCTTACCCG	CTTTGCTGAA	GCCCTCAAGG
ACAACACTGT	GGTGAAGACG	TTCACTCTGG	CCAACACGCA
TGCCGACGAC	AGTGCAGCCA	TGGCCATTGC	AGAGATGCTC
AAAGCCAATG	AGCACATCAC	CAACGTAAAC	GTCGAGTCCA
ACTTCATAAC	GGGAAAGGGG	ATCCTGGCCA	TCATGAGAGC
TCTCCAGCAC	AACACGGTGC	TCACGGAGCT	GCCTTTCCAT

AACCAGAGGC	ACATCATGGG	CAGCCAGGTG	GAAATGGAGA
TTGTCAAGCT	GCTGAAGGAG	AACACGACGC	TGCTGAGGCT
GGGATACCAT	TTTGAAGTCC	CAGGACCAAG	AATGAGCATG
ACGAGCATT	TGACAAGAAA	TATGGATAAA	CAGAGGCAAA
AACGTTTGCA	GGAGCAAAAA	CAGCAGGAGG	GATACGATGG
AGGACCCAAT	CTTAGGACCA	AAGTCTGGCA	AAGAGGAACA
CCTAGCTCTT	CACCTTATGT	ATCTCCCAGG	CACTCACCCCT
GGTCATCCCC	AAAAGTCCCC	AAAAAAGTCC	AGACTGTGAG
GAGCCGTCCT	CTGTCTCCTG	TGGCCACACT	TCCTCCTCCT
CCCCCTCCTC	CTCCTCCTCC	CCCTCCTTCT	TCCCAAAGGC
TGCCACCACC	TCCTCCTCCT	CCCCCTCCTC	CACTCCCAGA
GAAAAAGCTC	ATTACCAGAA	ACATTGCAGA	AGTCATCAAA
CAACAGGAGA	GTGCCCAACG	GGCATTACAA	AATGGACAAA
AAAAGAAAAA	AGGGAAAAAG	GTCAAGAAAC	AGCCAAACAG
TATTCTAAAG	GAAATAAAAA	ATTCTCTGAG	GTCAGTGCAA
GAGAAGAAAA	TGGAAGACAG	<u>TTCCCGACCT</u>	<u>TCTACCCAC</u>
<u>AGAGATCAGC</u>	<u>TCATGAGAAT</u>	<u>CTCATGGAAG</u>	<u>CAATTCGGGG</u>
<u>AAGCAGCATA</u>	<u>AAACAGCTAA</u>	<u>AGCGGGTGGA</u>	<u>AGTTCAGAA</u>
<u>GCCCTGCGAT</u>	<u>GGGAACATGA</u>	<u>TCTTTAGAG</u>	<u>AGGATGCAGA</u>
<u>ACTGTTCACT</u>	<u>GGTATTACAT</u>	<u>GAAATGCATT</u>	<u>GTGAGATGTT</u>
<u>TCTTAAATAC</u>	<u>CTTCTTCAAT</u>	<u>TCAAAATGAT</u>	<u>CCCTGACTTT</u>
<u>AAAATAATC</u>	<u>TCACCCATTA</u>	<u>ATTCCAAAGA</u>	<u>GAATCTTAAG</u>
<u>AATCAATCAG</u>	<u>CATGTTTCTT</u>	<u>CTGTAATAT</u>	<u>GAAAATAAAT</u>
<u>TTCCTTTTTA</u>	<u>TGTCGT-poly (A) -, tail</u>		

Fig. 2

CAGCCTGCCA	CTTGCCTCCC	TGCCTGCTTC	TGGCTGCCTT
GAATGCCTGG	TCCTTCAAGC	TCCTTCTGGG	TCTGACAAAG
CAGGGACCAT	GTCTACCTTT	GGCTACCGAA	GAGGACTCAG
TAAATACGAA	TCCATCGACG	AGGATGAACT	CCTCGCCTCC
CTGTCAGCCG	AGGAGCTGAA	GGAGCTAGAG	AGAGAGTTGG
AAGACATTGA	ACCTGACCGC	AACCTTCCCG	TGGGGCTAAG
GCAAAAGAGC	CTGACAGAGA	AAACCCCCAC	AGGGACATTC
AGCAGAGAGG	CACTGATGGC	CTATTGGGAA	AAGGAGTCCC
AAAAACTCTT	GGAGAAGGAG	AGGCTGGGGG	AATGTGGAAA
GGTTGCAGAA	GACAAAGAGG	AAAGTGAAGA	AGAGCTTATC
TTTACTGAAA	GTAACAGTGA	GGTTTCTGAG	GAAGTGATAT
CAGAGGAGGA	GGAGGAGGAG	TCCCAGGAGG	AAGAGGAGGA
AGAAGACAGT	GACGAAGAGG	AAAGAACAAT	TGAAACTGCA
AAAGGGATTA	ATGGAAGTGT	AAATTATGAT	AGTGTCAATT
CTGACAACTC	TAAGCCAAAG	ATATTTAAAA	GTCAAATAGA
GAACATAAAT	TTGACCAATG	GCAGCAATGG	GAGGAACACA
GAGTCCCCAG	CTGCCATTCA	CCCTTGTTGA	AATCCTACAG
TGATTGAGGA	CGCTTTGGAC	AAGATTAAAA	GCAATGACCC
TGACACCACA	GAAGTCAATT	TGAACAACAT	TGAGAACATC
ACAACACAGA	CCCTTACCCG	CTTTGCTGAA	GCCCTCAAGG
ACAACACTGT	GGTGAAGACG	TTCAGTCTGG	CCAACACGCA
TGCCGACGAC	AGTGCAGCCA	TGGCCATTGC	AGAGATGCTC
AAAGCCAATG	AGCACATCAC	CAACGTAAAC	GTCGAGTCCA
ACTTCATAAC	GGGAAAGGGG	ATCCTGGCCA	TCATGAGAGC
TCTCCAGCAC	AACACGGTGC	TCACGGAGCT	GCGTTTCCAT
AACCAGAGGC	ACATCATGGG	CAGCCAGGTG	GAAATGGAGA

TTGTCAAGCT	GCTGAAGGAG	AACACGACGC	TGCTGAGGCT
GGGATACCAT	TTTGAACTCC	CAGGACCAAG	AATGAGCATG
ACGAGCATTT	TGACAAGAAA	TATGGATAAA	CAGAGGCAAA
AACGTTTGCA	GGAGCAAAAA	CAGCAGGAGG	GATACGATGG
AGGACCCCAAT	CTTAGGACCA	AAGTCTGGCA	AAGAGGAACA
CCTAGCTCTT	CACCTTATGT	ATCTCCCAGG	CACTCACCCT
GGTCATCCCC	AAAAC TCCCC	AAAAAAGTCC	AGACTGTGAG
GAGCCGTCCT	CTGTCTCCTG	TGGCCACACT	TCCTCCTCCT
CCCCCTCCTC	CTCCTCCTCC	CCCTCCTTCT	TCCCAAAGGC
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GCCCTGCGAT	GGGAACATGA	TCTTTAGAA	AGGATGCAGA
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TCTAAAATAC	CTTCTTCAAT	TCAAAATGAT	CCCTGACTTT
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AAACAATCAG	CATGTTTCTT	CTGTAAATAT	GAATAATAAT
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TTAATTTAAA	GATGCTCTTC	CTATCTGTGG	ATGTGTTGGT
AACTCCGAGT	TGTAATGAGT	TCATGAAATG	TGCTGTTATT
TTTGTAAATCT	CAATAAATGT	GGATTGAAGT	TTTTTCCTT

-poly (A) -tail

Fig. 3

CAGCCTGCCA	CTTGCCCTCCC	TGCCTGCTTC	TGGCTGCCTT
GAATGCCTGG	TCCTTCAAGC	TCCTTCTGGG	TCTGACAAAG
CAGGGACCAT	GTCTACCTTT	GGCTACCGAA	GAGGACTCAG
TAAATACGAA	TCCATCGACG	AGGATGAACT	CCTCGCCTCC
CTGTCAGCCG	AGGAGCTGAA	GGAGCTAGAG	AGAGAGTTGG
AAGACATTGA	ACCTGACCGC	AACCTTCCCG	TGGGGCTAAG
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AGCAGAGAGG	CACTGATGGC	CTATTGGGAA	AAGGAGTCCC
AAAAACTCTT	GGAGAAGGAG	AGGCTGGGGG	AATGTGGAAA
GGTTGCAGAA	GACAAAGAGG	AAAGTGAAGA	AGAGCTTATC
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CAGAGGAGGA	GGAGGAGGAG	TCCCAGGAGG	AAGAGGAGGA
AGAAGACAGT	GACGAAGAGG	AAAGAACAAT	TGAAACTGCA
AAAGGGATTA	ATGGAAC TGT	AAATTATGAT	AGTGTCAATT
CTGACAACTC	TAAGCCAAAG	ATATTTAAAA	GTCAAATAGA
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ACTTCATAAC	GGGAAAGGGG	ATCCTGGCCA	TCATGAGAGC
TCTCCAGCAC	AACACGGTGC	TCACGGAGCT	GCGTTTCCAT
AACCAGAGGC	ACATCATGGG	CAGCCAGGTG	GAAATGGAGA

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AAAAATAATC	TCACCCATTA	ATTCCAAAGA	GATCTTAAAG
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TTCTTTTTTA	TGTCGTGAGA	TTTGTATTGG	CAAGAAGCAG
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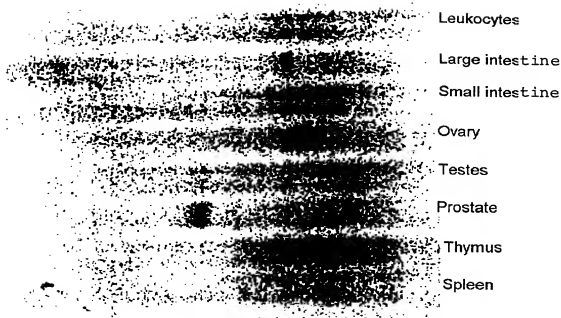
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- 46 -

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GTGAGTAAAT ACATCTGTCAG GTGTGTATGT AACATTACTG
GACATTAAAA AAAATTATTAC ATTCTC-poly (A) - tail

MSTFGYRRGL	SKYESIDEDE	LLASLSAEEL	KELERELEDI
EPDRNLPVGL	RQKSLTEKTP	TGTFSSREALM	AYWEKESQKL
LEKERLGECG	KVAEDKEESE	EELIFTESNS	EVSEEVYTEE
EEEESEQEEE	EEDSDEEERT	IETAKGINGT	VNYDSVNSDN
SKPKIEFSQI	ENINLTNGSN	GRNTESPAAI	HPCGNPTVIE
DALDKIKSND	PDTEVNLNN	IENITTQTLT	RFAEALKDNT
VVKTFSLANT	HADDSAAAI	AEMLKANEHI	TNVNVESNFI
TGKGILAIMR	ALQHNTVLTE	LRFHNRHIM	GSQVEMEIVK
LLKENTTLR	LYGHFELPGP	RMSMTSILTR	NMDKQORQRL
QEQQQEGYD	GGPNLRTKVW	QRGTPSSSPY	VSPRHSPWSS
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KGKKVKVKQPN	SILKEIKNSL	RSVQEKKMED	SSRPSTPQRS
AHENLMEAIR	GSSIKQLKRV	EVPEALRWEH	DL

09/4453/2



Spleen



Kidney

Skeletal muscle

Liver

Lung

Placenta

Brain

Heart

Fig. 5a

— 1.1 kb

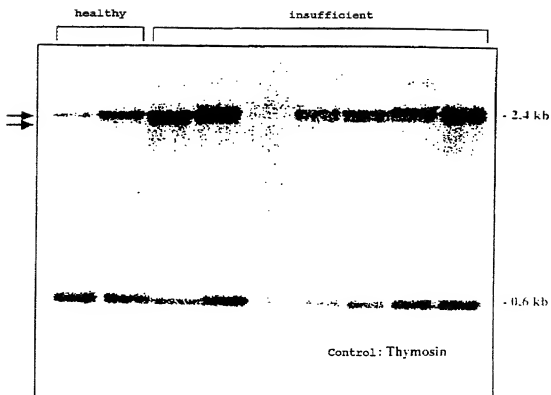
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09/445362

Fig. 5b



COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled MYOCARDIUM- AND SKELETAL-MUSCLE SPECIFIC NUCLEIC ACID, ITS PREPARATION AND USE, the specification of which

☐ is attached hereto.

☒ was filed on December 10, 1999 as Application Serial No. 09/445,362
and was amended on _____.

☐ was described and claimed in PCT International Application No. _____
filed on _____ and as amended under PCT Article 19 on _____.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose all information I know to be material to patentability in accordance with Title 37, Code of Federal Regulations, §1.56(a).

FOREIGN PRIORITY RIGHTS: I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

Country	Serial Number	Filing Date	Priority Claimed?
Germany	19725186.2	June 13, 1997	Yes
PCT	PCT/EP98/03584	June 15, 1998	Yes

PROVISIONAL PRIORITY RIGHTS: I hereby claim priority benefits under Title 35, United States Code, §119(e) and §120 of any United States provisional patent application(s) listed below filed by an inventor or inventors on the same subject matter as the present application and having a filing date before that of the application(s) of which priority is claimed:

Serial Number	Filing Date	Status

COMBINED DECLARATION AND POWER OF ATTORNEY

NON-PROVISIONAL PRIORITY RIGHTS: I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose all information I know to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56(a) which became available between the filing date of the prior application and the national or PCT international filing date of this application:

Serial Number	Filing Date	Status

I hereby appoint the following attorneys and/or agents to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith: Paul T. Clark, Reg. No. 30,162, Karen L. Elbing, Ph.D. Reg. No. 35,238, Kristina Bieker-Brady, Ph.D. Reg. No. 39,109, Susan M. Michaud, Ph.D. Reg. No. 42,885, Mary Rose Scozzafava, Ph.D., Reg. No. 36,268, James D. DeCamp, Ph.D., Reg. No. 43,580 (6)

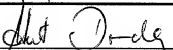
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Address all correspondence to: Karen L. Elbing, Ph.D. at Clark & Elbing LLP, 176 Federal Street, Boston, MA 02110.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

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